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Monte Carlo Simulation of Lipid Mixtures: Finding Phase Separation

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ABSTRACT The nonideal mixing of phosphatidylserine (PS) and phosphatidylcholine (PC) binary lipid mixtures was studied by computer simulation based on a model wherein the excess energy of mixing is divided between an electrostatic term and one adjustable term ΔE_m that includes all other nonideal interactions. The lateral distribution of the lipids and the energy of the mixtures were obtained by using Kawasaki relaxation in a canonical ensemble. The Gibbs free energies were calculated by Kirkwood's coupling parameter method. The simulation results are strongly dependent on simulation size for sizes smaller than about 1000 lipids. Nonideal interaction between lipids can result in large scale separation of lipid phases of different composition at reasonable ΔE_m values as well as clustering of like lipids. In plots of total Gibbs free energy of mixing versus PS mole fraction in PS/PC, the boundaries of the two phase region could be accurately determined. The electrostatic interaction influences cluster size and shape, and also the composition of phases in the two-phase region.

INTRODUCTION

Nonrandom mixing of biomembrane components has important implications in cell biology (Sweet and Schroeder, 1988; Thompson et al., 1992). In both simple model systems and in real biomembranes, descriptions of nonrandom mixing range over enormous and difficult-to-define scales of size and time (Wolf, 1992; Edidin, 1990; Kinnunen, 1991). Purely thermodynamic approaches enable the description of nonideal (i.e., nonrandom) mixing without explicit dependence on the size of clusters or "domains" (Tenchov, 1985; Lee, 1977). In contrast, computer simulations of biomembrane models enable a clear pictorial representation of the nonrandom mixing (Sperotto and Mouritsen, 1991; Lookman, 1982).

In a recent paper (Huang et al., 1993), we showed that the nonideal mixing of the negatively charged lipid PS and the zwitterionic lipid PC could be explored by comparing experimentally determined activity coefficients, γ_{exp} (Feigenson, 1989; Swanson and Feigenson, 1990), with activity coefficients, γ_{calc}, that were calculated from a computer simulation. The simulation is based upon a model wherein the excess energy of mixing is divided between an electrostatic term, U^{el}, and one adjustable term, ΔE_m, that includes all other nonideal interactions. After this earlier study, in order to compare mixing with and without electrostatic interaction, we performed a set of computer simulations on systems without, as well as with, electrostatic interaction. Our further work proves that the simulation actually does take the system to equilibrium, no matter what were the initial conditions. We also find that the equilibrium properties of the system strongly depend upon the size of the simulation. A key result from the earlier study was that reasonable values of ΔE_m should result in lateral separation of fluid bilayer phases that differ in PS/PC ratio. In the present study, we describe the calculated phase diagram for PS/PC mixtures and the simulation size effects on the phase diagram. We also explore the behavior of the computer simulation procedure as a one-phase region becomes more and more nonideal, and finally splits into two phases.

COMPUTER SIMULATION METHOD

Model for lipid mixing

Binary lipid mixtures are modeled as a two-dimensional triangular lattice. Each lattice site is occupied by a single lipid. The area of each site is 62 Å^2 (Nagle and Weiner, 1988). The total number of lipids (N), the number of PS (N_{PS}), and the number of PC (N_{PC}), are fixed for each simulation.

The total energy of a PS/PC mixture is given by (Huang et al., 1993):

$$U^T = \frac{ZN_{\text{PS}}U_{\text{PS-PS}}}{2} + \frac{ZN_{\text{PC}}U_{\text{PC-PC}}}{2} + N_{\text{PS-PC}}\Delta E_m + U^{\text{el}}(\chi_{\text{PS}}), \tag{1}$$

where $U_{\text{PS-PS}}$ and $U_{\text{PC-PC}}$ are the interaction energies for the designated lipid contacts, $N_{\text{PS-PC}}$ is the total number of PS/PC contacts in the lattice, Z is the number of nearest neighbors to a lattice site, which is 6 for a triangular lattice, $U^{\text{el}}(\chi_{\text{PS}})$ is the long-range electrostatic repulsion energy between PS molecules at PS mole fraction χ_{PS}, and ΔE_m is the nonelectrostatic excess mixing energy of a PS/PC pair, which is the only adjustable parameter used in our simulation.

The first two terms of U^T in Eq. 1 are independent of lipid distribution. Therefore they do not contribute to the nonideal mixing. The third term accounts for all nonelectrostatic contributions as nearest-neighbor interactions between PS and PC. The last term in Eq. 1 is the electrostatic energy of PS molecules. The detailed calculation of this term can be found in our earlier paper (Huang et al., 1993). Briefly, it utilizes a discrete charge theory for membranes developed by Sauvé and Ohki (Sauvé and Ohki, 1979) to account for the electrostatic repulsion between PS molecules. With their theory, the local electrostatic potential produced by an arbitrary arrangement of charged lipid can be calculated.

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In order to compare lipid mixtures with or without electrostatic charge, we also did a set of simulations with no electrostatic interaction. In this case, PS has no electrostatic charge, the last term in Eq. 1 is removed, and only the nearest-neighbor interaction is included in simulations.

Simulation of lipid lateral distribution

All simulations were carried out on a two-dimensional 100 × 100 triangular lattice, except for those aimed to study the finite size effect. A standard periodic boundary condition was used. For each simulation, N, χ_{PS}, ΔE_m, and T were held constant (i.e., a canonical ensemble). The Kawasaki relaxation method (Kawasaki, 1972; Jan et al., 1984) was used to bring the system to equilibrium: a lipid can interchange its position with that of a nearest neighbor with a probability of minimum (1, $\exp(-\Delta u)$), where Δu is the energy difference of the system due to the interchange ("lipid move").

Each simulation gives an energy of the mixture and a nonideality parameter ν (defined as $\nu = N_{PS\text{-}PC}(\text{observed})/N_{PS\text{-}PC}(\text{ideal})$). We first allow a mixture to relax to equilibrium, then the ensemble average of the energy of the mixture is obtained in 2,000–30,000 Monte Carlo steps, depending on how nonideal is the mixture. Typically, more Monte Carlo steps are needed as the system approaches a two-phase region. Each simulation was repeated independently at least eight times, and the average value of these replicates was used to calculate the Gibbs free energy.

Calculation of Gibbs free energy

Kirkwood's coupling parameter method (Kirkwood, 1935; 1936; Haile, 1986) was used to calculate the excess Gibbs free energy of the lipid mixtures (Huang et al., 1993),

$$\Delta G^E_{\text{mix}} = N_0\left\{\left[\int_0^1 \langle U^{\text{el}}(\chi_{\text{PS}})\rangle\, d\lambda_\psi - \chi_{\text{PS}}U^{\text{el}}(\chi_{\text{PS}}=1)\right]_{\lambda_E=0} \Big/ N + \int_0^{\Delta E_m} \langle N_{\text{PS-PC}}\rangle / N\, d\lambda_E \,\Big|_{\lambda_\psi=1}\right\} \quad (2)$$

where λ_E and λ_ψ are coupling parameters, $\langle\ \rangle$ represents ensemble averages, and N_0 is Avogadro's number. For mixtures with no electrostatic charge, only the second integral is used. The total Gibbs free energy of mixing is then

$$\Delta G^{\text{total}}_{\text{mix}} = \Delta G^E_{\text{mi}} + RT(\chi_{\text{PS}}\ln\chi_{\text{PS}} + \chi_{\text{PS}}\ln\chi_{\text{PS}}) \quad (3)$$

RESULTS AND DISCUSSION

Size effects and equilibrium test

A two-dimensional triangular lattice is often used in computer simulation to model lipid membranes (Mouritsen et al., 1983; Mouritsen, 1991; Ipsen and Mouritsen, 1988; Sperotto and Mouritsen, 1991; Zhang et al., 1992, 1993). The *general* effect of finite size on the simulation is well understood (Privman, 1990). However, finite size effects on each *particular* system must be examined. Therefore, we performed a series of simulations with various lattice sizes. The total number of lipids in the simulation, N, ranged from 100 to 10,000. We chose two neutral lipid mixtures of equal lipid fractions with ΔE_m = 0.4 or 0.5 kT. Both mixtures are in the one-phase region, but the latter is very close to the two-phase boundary. Fig. 1 shows the normalized energies of mixtures obtained from simulations as a function of $1/N$. We see that despite using periodical boundary conditions, *strong size dependence* was found for simulations with size smaller than about 1000 lipids. That is, the energy of a mixture increases as simulation size decreases.

The simulation lattice size limits the cluster size of like lipids. When the lattice size used is similar to or smaller than the largest cluster size, the energy and the lipid distribution obtained from simulation will be strongly dependent on the lattice size. Small simulation lattice size prevents big clusters from forming, and the extra interfacial regions due to small clusters result in *an overestimate of energy*. The mixture with ΔE_m = 0.5 kT is closer to the phase boundary and has larger cluster size, so it should show stronger dependence on simulation size than the mixture with ΔE_m = 0.4 kT. Fig. 1 shows that this indeed is the case. As the system crosses a phase boundary and the number of phases increases, *any* finite size simulation will eventually fail, because the size of new phases becomes infinite.

The Kawasaki relaxation method was used to bring the system to equilibrium. A meaningful ensemble average of a thermodynamic quantity can only be obtained after the system reaches equilibrium. This requirement becomes increasingly problematic as the mixture approaches a two-phase region, where simulations become inefficient (i.e., the number of Monte Carlo steps needed for each simulation in-

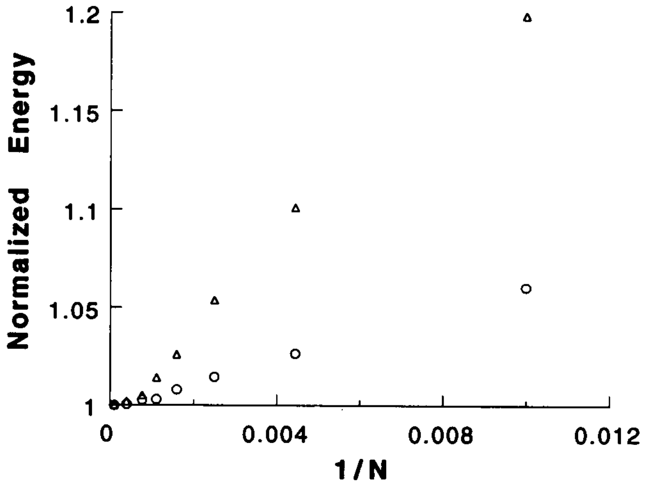

FIGURE 1 Normalized energy of lipid mixtures as a function of the inverse of simulation size N. N ranges from 100 to 10,000 lipids. Energies are normalized with that from the simulation of N = 10,000. The simulations were performed on binary mixtures of equal lipid fraction with nonideal interaction energy ΔE_m = 0.4 kT (○); 0.5 kT (△).

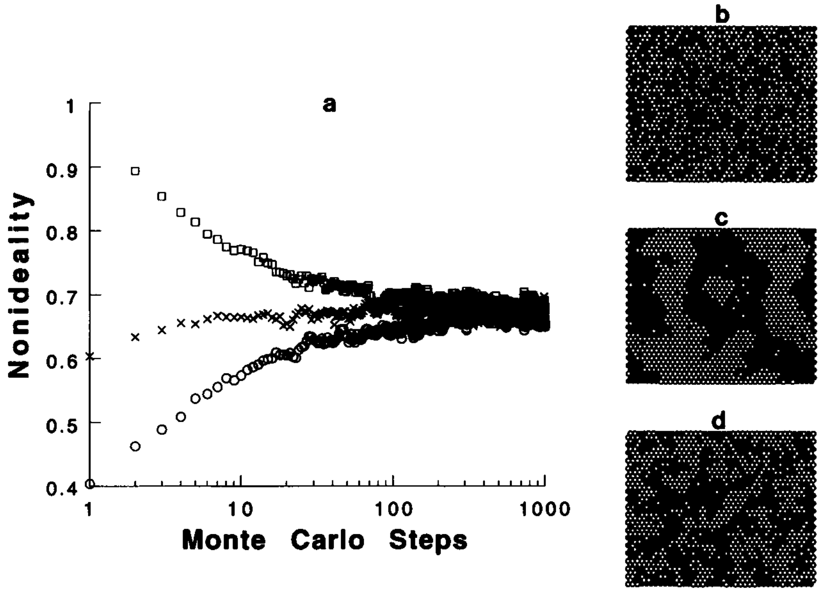

FIGURE 2 Equilibrium test for binary mixtures of equal lipid fractions with nonideal interaction energy $\Delta E_m = 0.4\ kT$. Electrostatic interaction of lipids was not included. (*a*) Nonideality parameter as a function of Monte Carlo Steps. Simulation starts from completely random mixing of two lipids (□); near equilibrium (×); from big clusters of lipids (○). (*b*–*d*) Lipid lateral distribution of initial states and final states. (*b*) The randomly mixed initial state; (*c*) the big-cluster initial state; (*d*) the final equilibrium state.

creases enormously when phase separation occurs). However, simple but crucial tests are sometimes neglected, and nonequilibrium results get into the literature. To see how many initial Monte Carlo steps were needed to relax a mixture to equilibrium (i.e., before taking the ensemble average), we ran the same simulation several times *with very different initial states*: (i) random mixing of two lipids; (ii) big clusters of the same lipid type; and (iii) a state near equilibrium. For the particular mixture shown in Fig. 2, we see that the Kawasaki method is able to quickly relax systems from the different initial states within about 100 Monte Carlo steps. However, the history of the initial state is not completely erased until about 500 Monte Carlo steps. The initial number of Monte Carlo steps we used ranged from 400 to 4000, depending on how nonideal a particular mixture is.

Phase behavior of lipid mixtures

The total Gibbs free energy was calculated for a given mixture composition and nonideal interaction energy ΔE_m according to Eqs. 2 and 3. Fig. 3 *a* and Fig. 4 show ΔG^T_{mix} vs. χ_{PS} for lipid mixtures *with* and *without* electrostatic interaction, respectively. Fig. 3, *b*–*d*, shows snapshots of the lateral distribution of lipids at several different values of ΔE_m. At low ΔE_m, mixing of two lipids is very favorable. ΔG^T_{mix} vs. χ_{PS} is concave upwards. The lateral distribution of

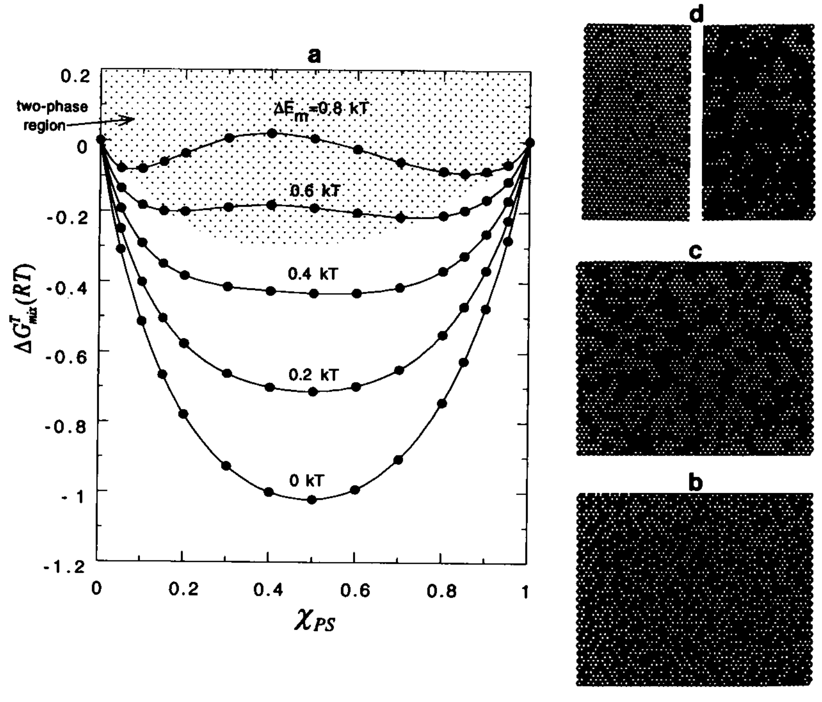

FIGURE 3 Phase behavior of lipid mixtures *with* electrostatic interaction. (*a*) The total Gibbs free energy of mixing, ΔG^T_{mix}, as a function of PS fraction, X_{PS}, and nonideal interaction energy, ΔE_m. The dotted area represents the two-phase region; (*b*–*d*) snapshot pictures of the lateral distribution of lipid mixtures with $X_{PS} = 0.5$ for various assumed PS-PC interactions. (*b*) $\Delta E_m = 0\ kT$, lipids distribute uniformly; (*c*) $\Delta E_m = 0.5\ kT$, PS and PC form clusters; (*d*) $\Delta E_m = 0.6\ kT$, phase separation occurs: the mixture splits up into two phases with $X_{PS} = 0.15$ and $X_{PS} = 0.75$, respectively. PS (●); PC (○).

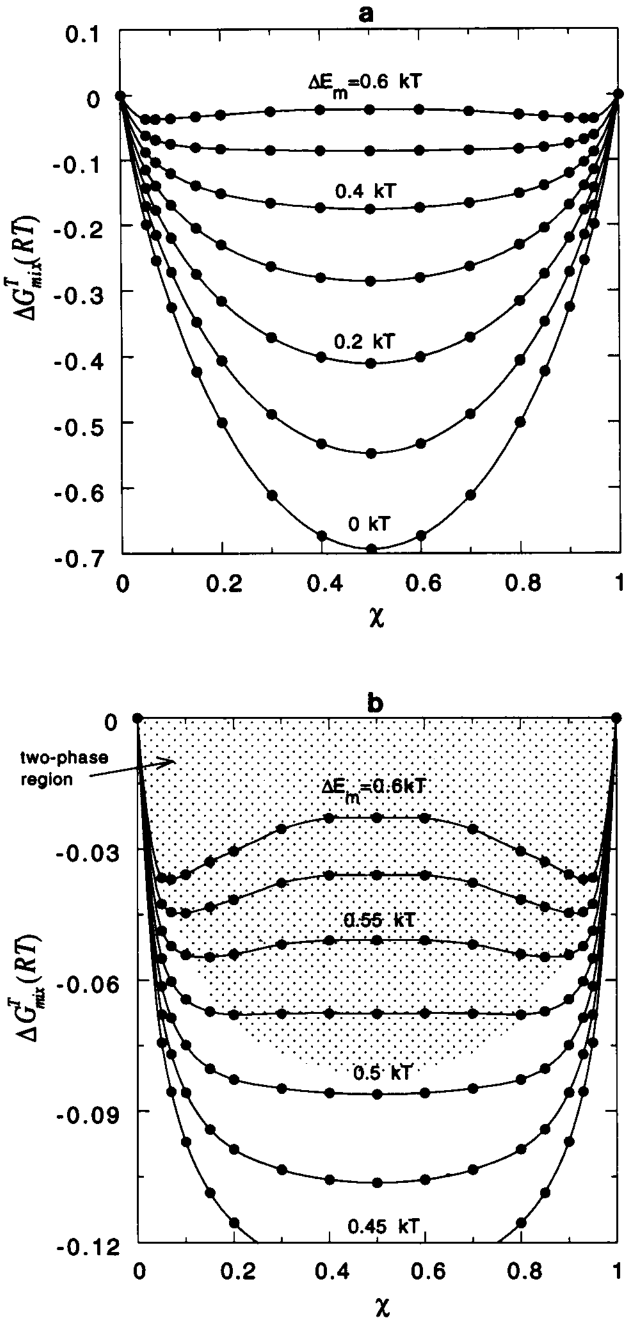

FIGURE 4 Phase behavior of lipid mixtures *without* electrostatic interaction. (*a*) The total Gibbs free energy of mixing, ΔG^T_{mix}, as a function of lipid fraction, χ_{PS}, and nonideal interaction energy, ΔE_{m}. (*b*) Enlarged to show phase boundaries. The dotted area represents the two-phase region.

lipids is uniform over the entire lattice at all χ_{PS} (Fig. 3 *b*). As ΔE_{m} increases, interactions between lipids cause clustering of like lipids, but the mixture stays as one homogeneous phase (Fig. 3 *c*). When ΔE_{m} is high enough, phase separation occurs: the mixture splits up into two phases with different lipid composition (Fig. 3 *d*). The dotted area in Fig. 3 *a* represents this two-phase region. Geometrically, when ΔG^T_{mix} becomes convex upwards at any lipid composition (i.e., $\partial^2 \Delta G^T_{\text{mix}}/\partial^2_\chi < 0$), phase separation occurs. The positions of the phase boundaries can be determined by the double tangent method (i.e., $\partial \Delta G^T_{\text{mix}}(\chi = a)/\partial \chi = \partial \Delta G^T_{\text{mix}}(\chi = b)/\partial \chi$, where *a* and *b* are phase boundaries), and the amount of material in each phase is given by the lever rule (Moore, 1962).

Fig. 3 *c* shows the lateral distribution of a lipid mixture with $\chi_{\text{PS}} = 0.5$ and $\Delta E_{\text{m}} = 0.5\ kT$. In this case, PS molecules form clusters despite the electrostatic repulsion. We note that the distribution shown in Fig. 3 *c* represents the *maximum possible nonideality (maximum clustering)* for this one-phase mixture. Based on our determination of the activity coefficients obtained from Ca^{2+}-PS binding experiments (Feigenson, 1989; Swanson and Feigenson, 1990), and our earlier computer simulation work (Huang et al., 1993), we believe that this calculated distribution is a good representation of the mixing behavior of (16:0, 18:1)PS/(16:0, 18:1)PC and (16:0, 18:1)PS/(14:1, 14:1)PC binary systems. The fact that these mixtures are so close to the two-phase region implies that phase separation can occur in real membranes composed of nonideally mixed lipids.

The nonelectrostatic excess mixing energy of a PS/PC pair, ΔE_{m}, in Figs. 3 and 4 is in units of kT. In order to view the phase behavior in the normal T vs. χ phase diagram, we need to choose a mixture with fixed ΔE_{m} value. For example, let $\Delta E_{\text{m}} = 0.55\ kT$ at 293 K, i.e., $\Delta E_{\text{m}} = 2.22 \times 10^{-21}$ J. A phase diagram is then constructed, using the data in Figs. 3 *a* and 4 *b* for this particular mixture. Fig. 5 shows the phase boundaries of such mixtures with and without electrostatic interaction. The regions under the boundaries are two-phase regions, and above are one-phase.

Comparing systems with and without electrostatic interaction (Fig. 5), an interesting similarity is that in both cases the two phase regions melt at temperatures above about 315 K (equivalent to $\Delta E_{\text{m}} < 0.5\ kT$ in Figs. 3 *a* and 4 *b*). The clear differences between the two systems are the shapes and positions of the phase boundaries. The phase boundary is symmetrical about $\chi = 0.5$ without electrostatic interaction. With

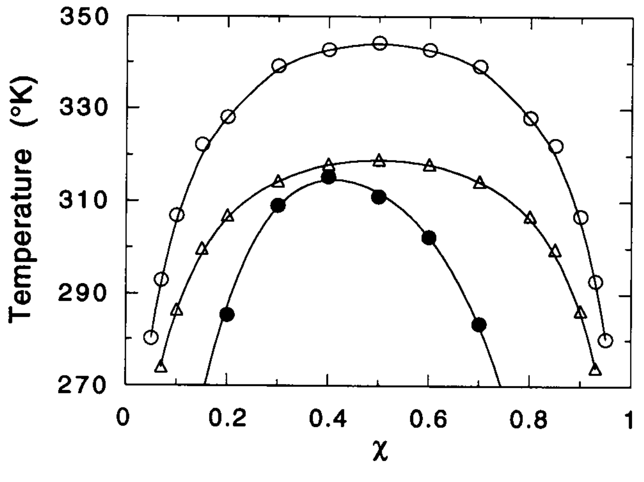

FIGURE 5 Phase diagram of a hypothetical lipid mixture with $\Delta E_{\text{m}} = 2.22 \times 10^{-21}$ J. The areas under curves are two-phase regions. Phase boundaries were calculated from simulations with size N = 10,000 and electrostatic interaction (●); with N = 10,000 and without electrostatic interaction (△); and with N = 100 and without electrostatic interaction (○).

electrostatic interaction the boundary shifts to lower PS concentration and lower temperature, and also the two-phase region is considerably narrower. This is a direct result of electrostatic repulsion between PS molecules. When phase separation occurs in a system with electrostatic charge, formation of a phase containing high concentration of charged-lipid is unfavorable.

We note that $\Delta G^T_{\mathrm{mix}}$ plotted in Figs. 3 and 4 as well as Eq. 3 does not include the (unknown) free energy difference of the two pure lipid states. However, that energy term is a linear function of the lipid composition. The positions of the phase boundaries *will not change* when a linear function is superimposed on $\Delta G^T_{\mathrm{mix}}$.

Finite size effect on phase behavior

Due to the finite size effect, in a two-phase region the canonical ensemble simulation cannot give a correct description of lipid lateral distribution and always overestimates the energy of such mixtures. Thus, we see in Figs. 3 *a* and 4 that the Gibbs free energy in the two-phase region is higher than that at the phase boundaries with the same ΔE_{m} value. This effect is more severe in systems with electrostatic charge (Fig. 3 *a*), since the electrostatic repulsion creates smaller clusters and more interfacial regions. For the same reason, the lateral distribution shown in Fig. 3 *d* was not obtained from a single simulation within a two-phase region. Instead, it was pieced together from two simulations, performed at each phase boundary.

In order to understand how the phase diagram depends on simulation size, we also performed the Gibbs free energy calculation with simulation size $N = 100$. Fig. 6 shows $\Delta G^T_{\mathrm{mix}}$ vs. χ for the same systems shown in Fig. 4 *b* but calculated with $N = 100$. Comparing Figs. 4 *b* and 6, we see that, for the smaller simulation size, (i) the values of $\Delta G^T_{\mathrm{mix}}$ are always higher; (ii) the free energy barriers between two minima of $\Delta G^T_{\mathrm{mix}}$ are higher; and (iii) some phase separations are predicted (e.g., $\Delta E_{\mathrm{m}} = 0.5\ kT$) that disappear in larger size simulations. Fig. 5 shows the phase boundaries generated from $N = 100$ simulations as well as those for the $N = 10{,}000$ simulations for the mixture discussed earlier, which has $\Delta E_{\mathrm{m}} = 2.22 \times 10^{-21}$ J. We see that $N = 100$ simulations predict higher transition temperatures than those from $N = 10{,}000$ simulations. For example, at $\chi = 0.5$, the transition temperature is about 24 K higher.

Since the calculated phase boundaries depend on the size of the simulation, it is important to find out whether $N = 10{,}000$ is large enough to accurately determine the phase boundaries. We note first that Fig. 1 shows that, for $N = 10{,}000$, any corrections are within the uncertainty of the simulation for the one-phase region. Second, the thermodynamic quantities in the limit of infinite lattice size can be estimated using finite size scaling theory (Binder, 1992; Siepmann et al., 1992; Privman, 1990). Since we had two sets of Gibbs free energy diagrams generated with two different simulation sizes, we could verify the phase boundaries using the method developed by Lee and Kosterlitz (Lee and Kosterlitz, 1990, 1991). Briefly, by watching how the barrier height between minima in $\Delta G^T_{\mathrm{mix}}$ vs. χ diagrams changes with simulation size N, the true ΔE_{m} values giving rise to phase separation can be determined. We found perfect agreement between our detection of minima in Fig. 4 *b* where $N = 10{,}000$ and barrier height comparison analysis according to Lee and Kosterlitz of Figs. 4 *b* and 6, in predicting phase separation.[1]

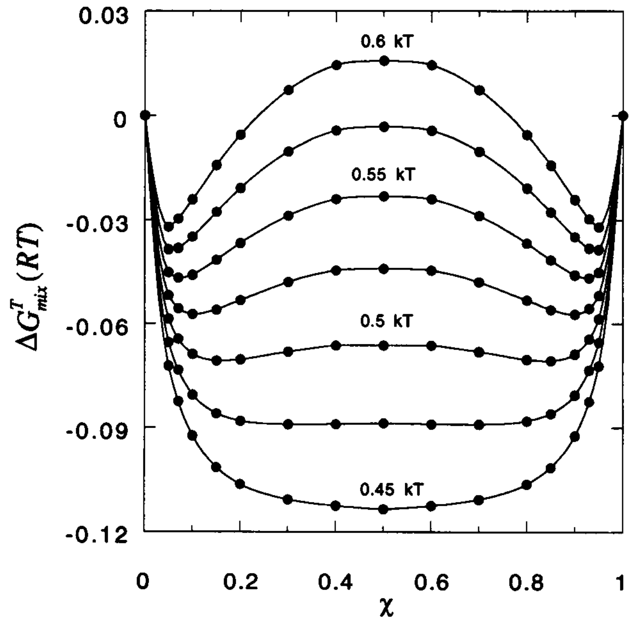

FIGURE 6 Phase behavior of lipid mixtures *without* electrostatic interaction calculated with simulation size $N = 100$. All the parameters are the same as in Fig. 4 *b* except that the simulation size is 100 times smaller.

Comments on the computer simulation method

We found that the Kawasaki relaxation method became inefficient for sampling the system phase space when large clusters form. This inefficiency can be reduced by using a cluster algorithm (Swendsen and Wang, 1987; Wang and Swendsen, 1990), which we will incorporate into our future simulation work.

Our original goal was to simulate the activity coefficients of lipids in binary mixtures. We used Kirkwood's coupling parameter method to calculate the Gibbs free energy of mixing from computer simulation. The advantage of this method is that the Gibbs free energy of mixing can be mapped exactly over the whole one-phase region, so that the activity coefficients of lipids can be calculated (Huang et al., 1993).

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[1] Lee and Kosterlitz (1990, 1991) used that part of the free energy which describes the composition dependence of the total free energy for their analysis. In our case, the Gibbs free energy of mixing was used. Note also that the free energy in their papers was defined as the free energy of all particles within the simulation box, which is equivalent to the quantity $\Delta G^T_{\mathrm{mix}}\ N/N_0$ in our notation. An application of this method for a bilayer mixture can be found in Zhang et al. (1993).

Although this approach is often used to calculate the free energy for lattice models, it may not to be the best method to determine the phase diagram: (i) to construct a phase diagram, we need to do many simulations to cover the whole one-phase region; and (ii) systems with little interface, such as in Fig. 4 *b*, have small barrier height, making precise determination of the phase boundary difficult. A better way to construct a phase diagram directly may be to use the recently developed Gibbs ensemble method (Panagiotopoulos, 1987; Panagiotopoulos et al., 1988). With this method, the interfacial region existing in a canonical or grand canonical ensemble simulation is completely eliminated, and the phase boundaries for a given ΔE_{m} value can be determined directly from a single simulation.

Seemingly reasonable computer simulations of mixtures can in fact be highly misleading without knowledge of the phase boundaries. For example, one could obtain a lateral distribution of lipids from a simulation without realizing that there are two coexisting phases in that region. The finite size effect would make that lateral distribution meaningless.

SUMMARY

1. Computer simulation study of a model binary lipid mixture shows that nonideal interaction between two types of lipids can result in large scale phase separation as well as microscopic clustering of like lipids, depending on the strength of the interaction.

2. The Gibbs free energy of mixing can be calculated from computer simulations for mixtures of PS and PC using Kirkwood's coupling parameter method. The boundary of the two-phase region can be recognized from $\Delta G^{T}_{\mathrm{mix}}$ vs. χ plots.

3. The finite size effect on simulation results was studied for the lattice model we used. In the one-phase region, there is strong size dependence for simulation size smaller than about 1000 lipids. Within the two-phase region, canonical ensemble simulation cannot produce the true lateral distribution or energy of lipid mixtures. Phase boundaries and $\Delta G^{T}_{\mathrm{mix}}$ can be determined accurately with simulation size of 10,000 lipids.

4. Comparison of mixtures with and without electrostatic interaction shows a profound influence on lipid mixing: electrostatic repulsion prevents formation of big clusters of PS molecules, creating more interface when phases separate, narrowing the two-phase region, and shifting the phase boundary to lower PS mole fraction.

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